data on mRNA expression showed that allograft cartilage tissues express cartilage specific markers.

Conclusions: The colonization of human viable knee and ankle allografts by recipient cells was investigated by genetic typing and mRNA expression. There is an evidence of persistence of donor cells particularly in knee allografts. This event is rare in ankle allografts, where the prevailing presence of host DNA suggests the ingrowth of recipient cells into the allograft, presumably migrating from the subchondral bone, in accordance with histological findings. The observed synthesis of cartilage specific RNAs in some of the analysed samples argues for the acquisition of a chondrocyte-like phenotype by some of these cells.

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HYMOVIS™, A HEXADECYLAMIDE HYALURONAN DERIVATIVE (HYADD®4-G), INHIBITS GENE EXPRESSION CHANGES INDUCED BY INTERLEUKIN-1β IN CHONDROCYTES AND SYNOVIAL FIBROBLASTS DERIVED FROM OSTEOARTHRITIS PATIENTS

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Osteoarthritis (OA) involves pathological change in all joint tissues, including cartilage degradation and synovitis. Synovial changes are associated with pain, while cartilage loss is a major indication for joint replacement. Cycloxygenase-2 (PTGS2) and inflammatory cytokines (IL6, TNF) are implicated in pain and increasing degradative enzymes and cartilage breakdown. A Cochrane study suggested positive clinical benefits of intra-articular hyaluronan (HA), despite its brief joint residency time. An amide-derivative of HA (HYMOVIS™) with increased joint retention was superior to native HA in improving gait, reducing synovial hyperplasia and cartilage *MMP13* expression in a sheep OA model. To elucidate the mechanism of the improved in vivo effects with the chemically modified HA, the effects of HYMOVIS™ on chondrocytes and synoviocytes from OA patients were evaluated.

Chondrocytes (HAC, n = 6) and synoviocytes (HSF, n = 6) were isolated from OA patients at the time of knee replacement. HYMOVIS™ or native HA (0, 0.5, 1.0 or 1.5 mg/mL) was added to primary HAC or early passage HSF with interleukin-1β (IL1β, 2 ng/mL). Cultures were terminated 30 minutes later for Bioplex® quantitation of phosphoproteins (p-JNK, p-NFκB, p-p38), or 24 hours later for RNA isolation and analysis of gene expression by real time RT-PCR, and measurement of MMP13 activity in the media. Only statistically significant results are reported.

In both HAC and HSF IL1β increased expression of *MMP1, MMP13*, *PTGS2, IL6* (>100fold), all phosphoproteins (3–10fold) and MMP13 activity. Cell specific effects were seen with IL1β increasing expression of *ADAMTS4* in HAC; *ADAMTS5* in HSF (~10fold); and causing a 2–3fold reduction of mRNA for *COL2A1* and *ACAN* in HAC and *COL1A1* in HSF.

In both cell types, HYMOVIS™ added with IL1β decreased *MMP13*, *ADAMTS4, ADAMTS5, PTGS2, IL6* expression, normalized matrix protein expression, but had no effect on phosphoproteins. Cell-type specific effects included decreased *MMP1* expression only in HSF, and reduced MMP13 activity only in HAC. In HAC, HYMOVIS™ preincubation was superior to simultaneous addition in reducing *ADAMTS, MMP*, *PTGS2*, and *IL6* expression, but it also inhibited expression of *TIMP1* and *TIMP3*, and abrogated the rescue of *COL2A1* and *ACAN* expression. There was a less dramatic effect of HYMOVIS™ preincubation on gene expression in HSF compared with HAC.

These results demonstrated a significant beneficial effect of the HA derivative, HYMOVIS™, on both synoviocyte and chondrocyte metabolism in vitro. This suggests HYMOVIS™ may have broader OA disease modifying actions and clinical benefit by reducing the negative effects of degradative enzymes and inflammatory cytokines in comparison to native HA.

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EFFECTS OF SURGERY AND POST-OPERATIVE INTRA-ARTICULAR CORTICOSTEROIDS ON SYNOVIAL FLUID COLLAGEN BIOMARKERS IN AN EQUINE MODEL OF OSTEOARTHRITIS

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Purpose: Little is known about collagen metabolism in the joint after surgery and how anti-inflammatory drugs affect this metabolism. The purpose of this study was to investigate the effects of arthroscopic surgery and post-surgical intra-articular administration of triamcinolone acetonide (TA) on collagen synovial fluid biomarkers using an animal model of osteoarthritis. We hypothesized that collagen synthesis and degradation would increase in synovial fluid after arthroscopic removal of osteochondral fragments, and that there would be greater collagen degradation in the TA treated group than in saline controls.

Methods: In 7 normal adult Quarter Horses an osteochondral fragment was arthroscopically created on the dorsal medial aspect of the first phalanx in one randomly chosen metacarpophalangeal joint (MCPJ). MCPJ synovial fluid was collected on weeks 0 (fragment creation), 16 (fragment removal), 17, 18, and 20. After fluid collection on week 17, horses were divided into 2 treatment groups: (1) horses (n = 4) that received 1 mL TA (10 mg) and (2) horses (n = 3) that received 1 mL saline injected into the MCPJ from which the fragment was removed. Effects of surgery and TA treatment were evaluated in week 18 and 20 samples. Injured MCPJ synovial fluid CPII, C12C, C2C (IBEX Technologies), and CTXII (IDS/Nordic) concentrations were evaluated using commercially available ELISAs previously validated for equine use. Synovial fluid biomarker concentrations for all weeks were compared to each other using unpaired t-tests. $P < 0.05$ was considered significant.

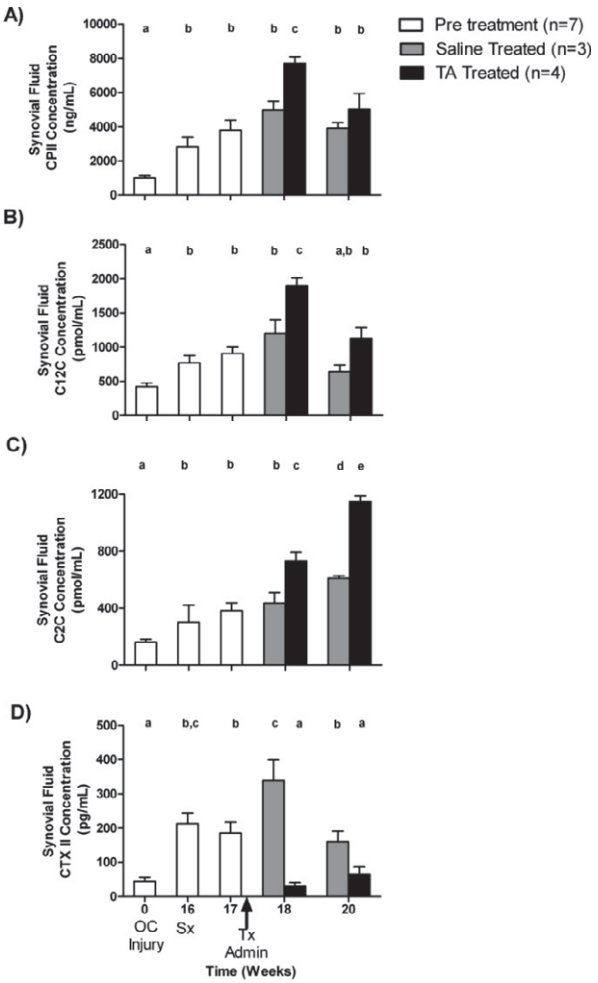

Fig. 1. Mean±SD synovial fluid biomarker concentrations for CP II (A), C12C (B), C2C (C), and CTX II (D). Week 0 = osteochondral (OC) fragment creation; week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection (arrow – Tx Admin) of either triamcinolone acetonide (TA) or saline; week 18 = 2 weeks post OC fragment removal and 1 week post TA (black bar) or saline (gray bar) injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post TA (black bar) or saline (gray bar) injection. Different letters indicate significance differences ($P < 0.05$).

Results: Sixteen weeks after creation of an osteochondral fragment, concentrations of CPII, C2C, C12C, and CTX II all significantly increased